IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
:
Yuki Katayama, et al.)
: Group Art Unit: 1657
Serial No. 10/531,315)
: Examiner: Amanda P. Wood
Filed: April 13, 2005)
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For: METHOD FOR QUANTITATIVELY DETERMINING CHOLESTEROL IN HIGH-DENSITY LIPOPROTEIN AND REAGENTS THEREFOR
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DECLARATION

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Yuki Katayama of 905 SURPASS MISHIMAHONCHO, 1-10, Honcho, Mishima-shi, Sizuoka, Japan do declare as follows:

I finished my bachelor course of Agricultural Sciences, Faculty of Agriculture, Okayama University in March, 1995, and I was given the degree of B.A. I finished my master course at Graduate School of Agriculture, Okayama University in March, 1997, and I was given the degree of M.A.

Since April, 1997, I have been employed by KYOWA MEDEX CO., LTD.

Since April, 1997, I have been engaged in the research on development of diagnostic reagent kits, mainly related to lipids (e.g. HDL cholesterol).

In the Experiments shown below, I used the following reagents and enzymes.

HEPES (manufactured by BDH Laboratory), EMSE (manufactured by Daito Chemix Corporation), sodium dextran

sulfate (molecular weight: 500,000) (manufactured by Pharmacia), bovine serum albumin (BSA; manufactured by Oriental Yeast), Nymeen L207 (polyoxyethylene dodecylamine; manufactured by NOF), Pionin D3110 (polyoxyethylene laurylamine; manufactured by Takemoto Yushi), Newcol OD420 (polyoxyethylene octadecylamine; manufactured by Nippon Nyukazai), BLAUNON 209 (polyoxyethylene oleylamino ether; manufactured by Aoki Yushi), Emulgen 430 [polyoxyethylene oleylether (HLB16.2); manufactured by Kao], Nikkol BO-10TX [polyoxyethylene oleylether (HLB14); manufactured by Kao], Morinol O-200 [polyoxyethylene oleylether (HLB15.3); manufactured by Morin Chemical Industries Co., Ltd.], Morinol O-300 [polyoxyethylene oleylether (HLB16.6); manufactured by Morin Chemical Industries Co., Ltd.], 4-aminoantipyrine (manufactured by Saikyo Kasei), Peroxidase (manufactured by Toyobo), LPL6 (cholesterol esterase; manufactured by Amano enzyme), COO321 (cholesterol oxidase; manufactured by Toyobo).

Kits for quantitatively determining HDL cholesterol

Kits for quantitatively determining HDL cholesterol comprising the following first and second reagents were prepared. Table 1 shows the list of Detergent A in the First reagent of each of the Kits.

First reagent	
HEPES (pH7.5)	10 mmol/L
EMSE	0.3 g/L
sodium dextran sulfate (molecular weight: 500,000)	1.0 g/L
BSA	2.0 g/L
Detergent A	0.07 g/L
Second reagent	
HEPES (pH7.0)	10 mmol/L
4-Aminoantipyrine	0.3 g/L
Peroxidase	20 kU/L
LPL6	0.05 kU/L
COO321	3.0 kU/L

Table 1

Kits	Detergent A	
A1	Nymeen L207	polyoxyethylene dodecylamine
A2	Pionin D3110	polyoxyethylene laurylamine (=polyoxyethylene dodecylamine)
B	Newcol OD420	polyoxyethylene octadecylamine
C	BLAUNON 209	polyoxyethylene oleylamino ether (=polyoxyethylene oleylamine)
a1	Emulgen 430	polyoxyethylene oleylether (HLB16.2)
a2	Nikkol BO-10TX	polyoxyethylene oleylether (HLB14)
a3	Morinol O-200	polyoxyethylene oleylether (HLB15.3)
a4	Morinol O-300	polyoxyethylene oleylether (HLB16.6)

Quantitative determination of HDL cholesterol

HDL cholesterol in 30 samples of human serum samples were measured on Hitachi 7170 autoanalyzer, using the Kit A1.

(1) Preparation of calibration curve

A calibration curve showing the relation between HDL cholesterol concentration and "absorbance" was prepared by the measurement on Hitachi 7170 autoanalyzer using a physiological brine (HDL cholesterol concentration: 0.0 mg/dL) and serum (HDL cholesterol concentration: 60.0 mg/dL) as standard solutions.

"Absorbance" used herein means a value obtained by subtracting E1 from E2 on the basis of the two absorbances (E1 and E2) measured in the following reaction.

A standard solution (3 mL) and the first reagent (0.24 mL) were added to a reaction cell and the mixture was heated at 37°C for 5 minutes. After measurement of absorbance (E1) of the reaction mixture at a main wavelength of 600 nm and a sub-wavelength of 700 nm, the second reagent (0.08 mL) was added to the reaction mixture and the mixture was heated at 37°C for 5 minutes. Absorbance (E2) of the last reaction mixture was measured at a main wavelength of 600 nm and a sub-wavelength of 700 nm.

(2) Calculation of "absorbance" for a human serum sample by the reaction of the sample with the Kit A1

The same method as in the calculation of "absorbance" in (1) was carried out except that human serum sample was used instead of the standard solution used in the preparation of a calibration curve in (1) whereupon "absorbance" for the sample was calculated.

(3) Determination of HDL cholesterol concentration in a human serum sample

HDL cholesterol concentration in each sample was determined by correlating the "absorbance" calculated in (2) and the calibration curve prepared in (1).

The same operation as above was carried out except that each of the Kits A2, B, C and a1~a4 was used instead of the Kit A1, whereupon HDL cholesterol concentration of each sample of the 30 human serum samples was determined.

In the meanwhile, HDL cholesterol concentration of each sample of the 30 human serum samples was determined according to a DCM (a Designated Comparison method) mentioned in Clinical Chemistry, vol. 45, No.10, p. 1803-1812 (1999), and the values to be measured were compared with those obtained by the measurements using each of the Kits A1~A2, B, C and a1~a4.

Correlation coefficients between each of the values to be measured for each of the measurements and those for the DCM are shown in Table 2.

Table 2

Kits	Detergent A	Correlation Coefficient
A1	Nymeen L207	0.9610
A2	Pionin D3110	0.9068
B	Newcol OD420	0.9695
C	BLAUNON 209	0.8752
a1	Emulgen 430	0.6875
a2	Nikkol BO-10TX	0.6692
a3	Morinol O-200	0.5120
a4	Morinol O-300	0.6039

As shown in Table 2, it was proved that the measurement using the Kits of present claim 1 of the present invention, such as Kits A1 and A2 comprising polyoxyethylene dodecylamine, Kit B comprising polyoxyethylene octadecylamine, Kit C comprising polyoxyethylene oleylamine shows better correlation with measurement by DCM than measurement using the Kits a1~a4 comprising polyoxyethylene oleyl ether of Miki et al.

The undersigned declarant declares further that all

statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 30th day of October, 2008.

Yuki Katayama